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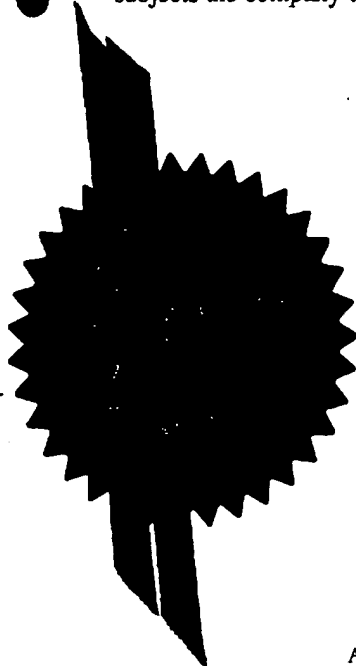
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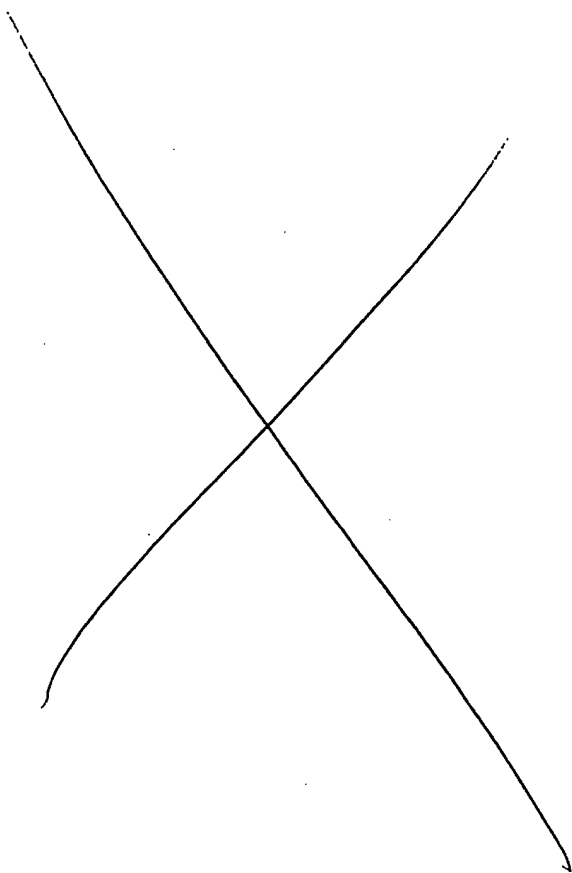
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Signed

*Andrew Gersey*

Dated 29th June 1998



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04JUN97-E278958-1 602904  
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The Patent Office

Cardiff Road  
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1. Your reference

SAMPLE

04 JUN 1997

2. Patent application number

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9711395.5

3. Full name, address and postcode of the or of each applicant (underline all surnames)

ENVIRONMENTAL SENSORS LTD  
DOWNHAMS HOUSE  
DOWNHAMS LANE  
CAMBRIDGE. CB4 1XT.

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

UNITED KINGDOM. 6207757002

4. Title of the invention

IMPROVEMENTS TO ELECTRODES FOR THE  
MEASUREMENT OF ANALYTES IN SMALL SAMPLES

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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EC2M 7LH

Patents ADP number (if you know it)

65177 2/498

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number  
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Date of filing  
(day / month / year)

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7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing  
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N/A.

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

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  - b) there is an inventor who is not named as an applicant, or
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Priority documents

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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

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Request for preliminary examination and search (Patents Form 9/77)

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11. I/we request the grant of a patent on the basis of this application.

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*Shull*

Date 30 May 1997

12. Name and daytime telephone number of person to contact in the United Kingdom

DR. S.C. WILLIAMS

Tel: (0223) 424225.

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***TITLE***

Improvements to electrodes for the measurement of analytes in small sample volumes.

***TECHNICAL FIELD***

This invention relates to new methods for the fabrication of electrode materials, the convenient construction of electrode devices from said electrode materials which are capable of accepting small volumes of samples, and a test method for the detection and quantification of a test species present in a small sample volume using said electrode devices.

***BACKGROUND***

The prior art contains many references to devices capable of accepting small volumes of sample material and interrogating analytes present in said sample either by optical or electrical analytical processes. In particular, the use and construction of sample chambers capable of filling by capillary action has been described previously in both the patent and scientific literature. Such devices may consist of electrodes deposited on a non-conducting substrate and coated with a reagent system specific for the analyte of interest and housed within a cavity whose dimensions are sufficiently small to allow introduction of a sample by capillary action and retention of the sample in close proximity to the electrodes, the electrodes being configured in such a way as to facilitate the measurement of specific electrical properties of the sample.

Such devices suffer from numerous drawbacks:

They are complex and multilayer in construction and are thus expensive to produce. The devices essentially consist of 4 elements, a first substrate material, conductive electrodes deposited on the first substrate material and coated with reagents, a spacer layer of defined thickness adhered to the substrate material using a pressure sensitive adhesive, and a second substrate material which is adhered to the spacer layer, again usually by a pressure sensitive adhesive.

The requirement to control the dimensions of the capillary fill cavity within very tightly defined manufacturing limits to allow capillary action to draw sample into the cavity. Exceeding these manufacturing tolerances will prevent the sample from entering the cavity by capillary action. Thus if the spacer is either too thick or too thin, the device will not fill with sample.

When viscous sample fluids such as blood are introduced to the capillary fill cavity, the chamber will fill with sample relatively slowly, thus compromising the ability to rapidly place the sample in contact with the measuring electrodes, and delaying the time taken to completed the analysis.

Variations in sample viscosity and thus sample surface tension characteristics result in variations of device fill time; this not only compromises the overall analysis time, but more importantly leads to imprecision in the analytical result since the time over which the sample is exposed to the analyte specific reagents resident within the cavity, is subject to variation.

### ***ESSENTIAL TECHNICAL FEATURES***

According to the present invention, there is provided a device which is capable of measuring electrochemically, levels of analytes present is a small fluid sample volume, consisting of a conductive layer coated with an analyte specific chemistry and deposited on a non-conducting substrate, a spacer layer deposited onto the non-conducting substrate by thick film printing, a monofilament mesh material coated with a surfactant and or a chaotropic reagent, said mesh being overlaid onto the spacer layer, and a second non-conductive substrate adhered to the mesh layer. The device is thus multilayer in construction and essentially consists of two surfaces separated by a printed spacer layer and a mesh, forming a cavity which is open at one end for the introduction of sample. The mesh material extends beyond the second substrate and forms a sample application area.

The conductive layer consists of graphite particles (in the size region 1 - 20  $\mu\text{m}$ , with a surface area in the range 1 - 50  $\text{m}^2/\text{g}$ ), and carbon particles (in the size region 5 - 70 nm, with a surface area of less than 150  $\text{m}^2/\text{g}$ ), held sufficiently close together to facilitate electrical contact between the particles, by a polymer binder. The polymer binder is chosen from a diverse range of suitable materials, and may be classified as either thermoset or thermoplastic in nature. The conductive layer is coated with a reagent which is specific to the analyte of interest.

The mesh material is interposed between the spacer layer (on the first substrate) and the second substrate, and functions to reduce the surface tension and or viscosity of the sample by virtue of the wetting agent coated onto its surface. Application of sample to the extended portion of the mesh, results in dissolution of the mesh coating material into the sample, reducing sample surface tension and allowing sample to flow into the device cavity. Sample will not enter the device cavity in the absence of a wetting reagent coated onto the mesh. Alternatively, in complex samples such as blood, where the measurement of a specific analyte is adversely affected by the presence of whole cells for example by poisoning an electrode surface, the mesh may be coated with an agent which lyses the cells on contact; this has the added advantage of reducing sample viscosity at the same time, whilst removing the whole cell interference.

The electrode devices produced in this way are characterised in that they allow the application of a small volume of sample (typically less than 2 microlitres) to the mesh extension, followed by flooding of the device cavity with sample, bringing it into intimate contact with the measuring electrodes.

The system may be deposited as a single electrode, a micro-electrode or as a microelectrode array. The electrode may be used in conjunction with reference/counter electrodes deposited on the same substrate.

In the use of devices according to the invention, the cavity is filled either by placing a drop of sample liquid on top of the exposed mesh at the edge of the cavity or by contacting the edge of the cavity with the sample.

### ***DESCRIPTION OF THE INVENTION***

According to the present invention, there is described a method for producing and using an electrode device for measuring analytes in small volumes of sample which consists of a) depositing a conducting layer of carbon and graphite, in a polymer binder on a first non-conducting substrate, b) depositing a second conducting layer consisting of silver/silver chloride to function as a reference/counter electrode, adjacent to but not continuous with the first conducting layer, c) coating the surface of the first conductive layer with a reagent or mixtures of reagents which react specifically with an analyte or analytes in a sample material, d) forming a spacer layer by thick film printing on top of the first non-conducting substrate and on top of the first conducting layer, in order to leave a portion of both the first and second conducting layers exposed, e) locating a coated mesh material on top of the spacer layer and permanently securing it to the spacer layer, f) locating a second non-conducting substrate on top of the mesh material and permanently securing it in such a way as to leave an extended area of mesh exposed, g) applying a sample to the extended mesh area in order to fill the cavity by wetting of the mesh with sample, and h) quantifying the analyte in the sample by reaction with reagents present on the first conducting layer.

The non-conducting substrate material may be a polyester sheet material polycarbonate, polyvinyl chloride, high density polypropylene or low density polypropylene. In a preferred embodiment, the polyester sheet material is heat stabilised prior to application of the conducting layers, to confer dimensional stability on the polyester material prior to processing.

The conducting layer which contains the graphite, carbon and polymer binder is characterised as follows:

The graphite component has an average particle size of less than 20  $\mu\text{m}$  and a typical surface area less than 50  $\text{m}^2/\text{g}$ , and is inherently conductive; it may be derived from either natural sources or produced synthetically.

The carbon component has an average particle size less than 1  $\mu\text{m}$ , and a typical surface area of less than 150  $\text{m}^2/\text{g}$ . Like the graphite component, it is also inherently conductive.

The polymer binder may be derived from any of the diverse number of polymer families, including but not limited to vinyl chloride, vinyl acetate, vinyl alcohol (and copolymers of vinyl chloride, acetate and alcohol), hydrocarbons, ethyl and methyl celluloses, epoxys, polyesters, alkyds, and a range of other

polymers containing functional reactive groups such as carboxylates, hydroxyls, amines, thiols, esters, epoxides and amides which enable the polymer to be cross linked.

The conducting electrode material may be deposited on the non-conducting substrate by a conventional printing process, including but not limited to thick film printing (also known as screen printing), lithography, letter press printing, vapour deposition, spray coating, ink jet printing, laser jet printing, roller coating or vacuum deposition.

Following deposition of the conductive electrode material, the polymer binder may be stabilised or cured by a number of conventional processes, including but not limited to forced air drying, forced air drying at elevated temperatures, infra-red irradiation, ultraviolet irradiation, ion beam irradiation or gamma irradiation. All of these processes result to varying degrees in the cross linking of individual molecules of the polymer binder.

The use of ultraviolet radiation requires the inclusion of a photo-sensitising reagent in the conductive electrode material, to initiate the polymer cross linking reaction.

The reagent chemistry located on top of the first conductive layer is characterised in that it contains all the components in a solid state necessary for measuring the concentration of analyte in a sample.

Such components include but are not limited to enzymes, enzyme cofactors, coenzymes, co-substrates, antibodies or other analyte binding partners, DNA or RNA, redox partners, buffers, ionophores and salts.

The reagent chemistry may also contain support matrices, binders and stabilisers for the other components, said matrices including but not limited to particles of graphite, carbon, silica, glass, latex, polyvinyl chloride, said binders including but not limited to polyvinyl alcohol, polyvinyl acetate, polyvinyl pyrrolidone, proteins, cellulose, cellulose acetate, and said stabilisers including but not limited to alcohols, esters, proteins, protein hydrolysates and both simple and complex carbohydrates.

The reagent chemistry may consist of a number of individually applied layers, each containing specific components, and its composition is such that it undergoes at least partial dissolution when contacted by the fluid sample.

The reagent chemistry may be deposited on the first conducting layer by a conventional deposition process, including but not limited to thick film printing (also known as screen printing), lithography, letter press printing, vapour deposition, spray coating, ink jet printing, laser jet printing, roller coating or vacuum deposition. Combinations of these deposition processes may be used to construct a multilayer chemistry phase. Following deposition of the reagent chemistry (or after deposition of each individual layer), the layer may be stabilised or cured by a number of conventional processes, including but not



limited to forced air drying, forced air drying at elevated temperatures, infra-red irradiation, ultraviolet irradiation, ion beam irradiation or gamma irradiation. All of these processes result to varying degrees in the cross linking of individual molecules of the polymer binder.

The spacer layer is characterised as follows:

It is deposited on the first non-conducting substrate by conventional thick film deposition, and may be stabilised or cured by a number of conventional processes, including but not limited to forced air drying, forced air drying at elevated temperatures, infra-red irradiation, ultraviolet irradiation, ion beam irradiation or gamma irradiation. All of these processes result to varying degrees in the cross linking of individual molecules of the polymer binder. The thickness of the spacer layer is controlled via a number of parameters, including but not limited to printing conditions (pressure, speed, screen tension and emulsion thickness) and ink properties such as solids content and viscosity.

The mesh layer is characterised as follows:

It is a synthetic, monofilament, woven material made from materials including but not limited to polyester or nylon.

The mesh is coated with a surfactant material, a detergent or wetting or a lysing agent from a group including but not limited to fluorosurfactants, non-ionic surfactants, ionic surfactants, zwitterionic surfactants, saponin, and sodium cholate.

Electrodes fabricated by this method have several characteristics that make their use desirable for the measurement of analytes:

Devices require a very small sample volume, typically less than 2 microlitres. Samples may be whole blood, plasma, serum, interstitial fluid, sweat, or saliva.

When the sample fills the sample cavity, a very thin film of sample is spread across the surface of the deposited chemistry, maximising contact with said reagents, and enabling reagents to be dissolved in the sample rapidly. This allows rapid attainment of the steady state.

In a preferential embodiment of the device the cavity is positioned at the end or edge of the device. This device may be readily filled with sample by contacting the edge of the test strip with the sample. In another preferred embodiment, the cavity may be positioned 0 - 2 mm from the edge of the device, thus exposing an area of the test strip which may be scraped along a surface (such as a punctured area of skin) in order to collect the sample.

Electrodes fabricated by this method may be used for the analysis of :

Analytes/species which can be directly oxidised or reduced by the removal or addition of electrons at an electrode.

Analytes/species which can be readily converted by an enzyme or a series of enzymes, to a product which can be directly oxidised or reduced by the removal or addition of electrons at an electrode.

Analytes or species which can be converted to a product by an enzyme, with the concomitant oxidation or reduction of an enzyme cofactor; said cofactor may then be directly oxidised or reduced by the addition/removal of electrons.

Analytes or species which can be converted to a product by an enzyme, which is in intimate contact with the electrode surface, such that the enzyme is able to pass or receive electrons directly from the electrode.

#### **EXAMPLE**

In one specific embodiment of the invention given by way of example, the electrode device consists of:

a) a non-conducting polyester sheet material (125  $\mu\text{m}$ ) in thickness, onto which is deposited by way of a screen printing process, a conductive ink material consisting of a mixture graphite particles (average particle size 1  $\mu\text{m}$ , with a surface area of 15  $\text{m}^2/\text{g}$ ), conductive carbon particles (average particle size 40 nm, surface area 100  $\text{m}^2/\text{g}$ ), and a vinyl chloride/acetate copolymer binder in an organic solvent. After deposition of the conductive ink, solvents are removed in a forced air oven, whilst the application of elevated temperature initiates the chemical cross linking of polymer binder by the bifunctional amine.

b) a silver/silver chloride, screen printed reference/counter electrode, located adjacent to the conductive carbon layer on the polyester support.

c) a spacer layer, screen printed in such a way as to leave part of the conductive carbon electrode and all of the reference/counter electrode exposed.

d) a multilayer reagent mixture which is specific for the measurement of glucose in the sample consisting of :

i) a mediator for the enzyme cofactor, NADH, deposited onto the exposed conductive carbon/graphite layer from aqueous solution by pipetting, and dried to leave a film of mediator coated onto the conductive carbon/graphite layer.

ii) a second layer deposited by thick film printing followed by drying, consisting of a mixture of graphite,  $\text{NAD}^+$ , buffer salts, surfactants, stabilisers and rheology modifiers.

iii) a third layer deposited by pipetting followed by drying, consisting of an aqueous solution of glucose dehydrogenase (NAD dependent), buffer salts and stabilisers.

e) a surfactant coated monofilament mesh material, located on top of the spacer layer and secured by thick film deposition of a second spacer layer

f) a second non-conducting layer, comprising a 75 micron thick polyester tape material coated on one side with a pressure sensitive adhesive, positioned on top of the monofilament mesh in such a way as to leave an extended area of the mesh exposed to act as a sample application zone.

When a suitable potential difference is applied between the conductive carbon and the silver chloride reference electrodes, the electrode device can be used for the measurement of glucose in a sample of blood using standard electrochemical techniques, such as but not limited to, chronoamperometry. Glucose is converted to gluconolactone with concomitant conversion of  $\text{NAD}^+$  to NADH by the action of the  $\text{NAD}^+$ -dependent glucose dehydrogenase, and NADH is reoxidised to  $\text{NAD}^+$  by the mediator compound. The mediator compound is in turn reoxidised at the electrode surface, and the current produced is proportional to the concentration of glucose in the sample.

***TITLE***

Improvements to electrodes for the measurement of analytes in small sample volumes.

***ABSTRACT***

Electrode devices capable of responding electrochemically to the presence of an analyte in a small volume of sample, comprising a container or sample cell consisting of an electrically conducting layer of graphite and carbon in a polymer binder, on a non-conducting substrate, a spacer layer on top of the non-conducting substrate exposing a defined open area of the electrically conducting layer, a monofilament mesh material coated with a wetting agent on top of the spacer layer, a non-permeable layer placed on top of the mesh and secured in place, characterised in that a fluid sample will flow along the mesh filaments by wetting of the mesh surface and that the electrically conductive layer is coated with a chemical or electrochemical reagent which, when in contact with an analyte in a fluid sample, reacts in such a way as to generate an electrical signal representative of the concentration of analyte.

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